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Neopterin production in relation to COVID-19 in the Haut-Ogooué Province, Gabon

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Abstract

Background In sub-Saharan Africa, understanding of the immune process associated with the COVID-19 pandemic remains scarce. This study aimed to investigate the relationship between plasma neopterin concentrations and COVID-19 infection, focusing on changes over time and age-related changes in immune response.

Methods A retrospective case study was conducted during the first wave of COVID-19 from March to August 2020. Whole blood and associated symptoms and comorbidities were collected from patients of all ages and sexes. Concentrations of plasma neopterin were measured using a commercial competitive neopterin ELISA (Neopterin ELISA, IBL International GmbH, Germany).

Results We analyzed data for 325 patients: 38% ($n=124$) with COVID-19, and 62% ($n=201$) without COVID-19, as a control group. We found that plasma neopterin concentrations were significantly higher in the COVID-19 group (mean value 45.1 nmol/L (SD 19)) than in the control group (mean value 33.8 nmol/L (SD 13)) ($p=0.004$). In addition, neopterin levels decreased gradually over time in patients with COVID-19 ($p<0.001$). Moreover, ROC analysis found that the best cut-off value for diagnosing COVID-19 patients based on plasma neopterin levels was 38.85 nmol/L with 70% sensitivity and 82% specificity (AUC, 0.74 [0.69–0.82], $p<0.05$). We also found an increase in neopterin production with increasing age ($p<0.001$).

Conclusion Our findings contribute to our growing understanding of neopterin levels as a promising biomarker for the detection of COVID-19 cases in sub-Saharan Africa.

Keywords SARS-CoV-2, COVID-19, Neopterin, Biomarker, Age, Immunosenescence

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Introduction

The emergence of the novel coronavirus disease COVID-19, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has led to an unprecedented public health crisis worldwide [1]. This virus poses an ongoing threat to humanity, infecting millions of people and causing significant morbidity and mortality [1, 2]. Its spreads faster than other coronaviruses, such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) [1], contributing to the rapid worldwide spread of the virus [2]. SARS-CoV-2 infection is characterized by a wide spectrum of symptoms similar to influenza, including breathlessness, sore throat, and fatigue [1]. In most cases, the infection is mild, including fever, dry cough, and fatigue, or cases may be asymptomatic, but symptoms can progress to pneumonia, acute respiratory distress syndrome, and multi-organ failure in elderly patients and those with underlying cardiac and respiratory disorders [3].

When the COVID-19 pandemic began, few laboratories focused on the immunological aspects of SARS CoV-2 [4]. However, as the need to understand the crucial role of immune responses and associated mechanistic factors became apparent, there was a significant shift. As a result, immune responses during the COVID-19 pandemic have been investigated extensively [5, 6]. The immune response involves a complex interplay of different components of the immune system. For instance, individuals affected with SARS-CoV or MERS-CoV have dysregulated cytokine production from both innate and adaptive immunity [7]. In addition, elevated production of various pro-inflammatory cytokines such IL-2, IL-6, IL-7, TNF-a, and the formation of interferon gamma (IFN-γ), which activates monocytes and macrophages, have been reported as defense mechanisms against SARS-CoV-2 [8].

Extensive research has been conducted to inform the development of immunotherapies and COVID-19 vaccines, and to understand resistance to the virus and the pathogenesis of severe disease. These studies range from its clinical manifestations to potential biomarkers that reflect pathological development and are relevant in investigating immune changes with COVID-19 [9]. For instance, biomarkers have been used to treat and monitor patients with COVID-19 infection [10], identify patients at risk of disease progression, and predict disease severity [11]. For example, elevated levels of C-reactive protein (CRP) have been used to track the progression of COVID-19 in patients and are associated with worse prognosis [12].

Neopterin is a biomarker of the cell-mediated immune response and is synthesized from guanosine triphosphate (GTP) [13, 14]. Neopterin is produced and released by monocytes, macrophages and dendritic cells in response to interferon gamma (IFNγ) [15]. It is regarded as an early biomarker of the cellular immune response and is used to investigate the cell-mediated immune status with considerable sensitivity [16]. For instance, elevated neopterin levels were significantly higher in patients with viral infections including dengue fever virus [17], HIV infection, and influenza [15]; bacterial infections like pulmonary tuberculosis [15]; parasitic infections including helminth parasitism and malaria [18], and autoimmune diseases [19].

Neopterin has been extensively studied in relation to COVID-19. For instance, neopterin levels are significantly correlated with disease severity and poor clinical outcomes [20–22]. In addition, serum neopterin levels are higher in patients with severe COVID-19 compared to those with mild disease [21]. Elevated serum neopterin levels at the time of hospital admission have been proposed as a hallmark of severe COVID-19 and a predictor of fatal outcomes [23]. However, neopterin also exerts anti-inflammatory and antioxidant effects by suppressing NF-κB signaling and NLRP3 inflammasomes [24] and can be viewed as a protective factor in COVID-19 [24].

While much has been learned about cellular and humoral immunity in response to COVID-19 around the world, particularly how response to the virus and vaccines varies with age and other demographic information, there is a major gap in our understanding of these processes in sub-Saharan Africa. There is still a great deal interest in studying this relationship in Africa, especially as models predicted much higher levels of COVID-19 cases and associated mortality on the African continent [25], but numbers of reported cases are low. Among other factors affecting reporting, immunological processes may have contributed to limiting disease transmission.

We studied neopterin in relation to the development of Covid-19 in Gabon, in Central Africa. Our understanding of the immune process in the study area remains poor because few studies are available. Two studies have investigated the understanding of clinical features and humoral immunity in response to SARS-CoV-2 infection [26, 27], while another examined biochemical and haematological markers in COVID-19 cases [28]. Moreover, few data are available on genome sequencing of SARS CoV-2 [29], the epidemiology of the first cases [30] and the emergence of variants of concern [31, 32]. In this context, we investigate changes in plasma neopterin production over time of infection and for different ages.

Materials and methods

Study design and participants

A retrospective case study was conducted in the hospital designated for COVID-19 in the Haut–Ogooué province, namely the Centre Hospitalier Régional Amissa

Bongo (CHRAB), during the first wave of COVID-19 from March to August 2020. During the study period, the Centre Interdisciplinaire de Recherches Médicales de Franceville (CIRMF) initiated a COVID-19 surveillance programme, according to the guidelines of the Gabonese Ministry of Health, the WHO Regional Official for Africa and the African Centre for Disease Control (Africa CDC).

Surveillance consisted of daily collection of clinical specimens including oropharyngeal and nasopharyngeal swabs (of all age and sexes), from all patients of all sexes and ages attending CHRAB for diagnosis of COVID-19 infection by polymerase chain reaction (PCR), with blood samples collected in parallel for other tests. Patients with suspected cases of COVID-19 were either admitted to the CHRAB or instructed to remain at home to prevent possible transmission of the disease to the wider community. In addition, PCR tests were repeated in patients admitted with COVID-19 on days 10, 14, 21 and 28 after admission.

The surveillance included the collection of socio-demographic information (e.g., age, sex, place of residence, occupation), clinical data (e.g., symptoms, signs of illness such as loss of smell and/or taste, runny nose, fever and persistent cough, fatigue, abdominal pain, chest pain, shortness of breath, headache, muscle pain, diarrhea and associated comorbidities), medical history, travel history, and laboratory parameters.

Two groups of patients were defined according to the presence of COVID-19 infection. Patients who tested positive for SARS-CoV-2 via nasopharyngeal Reverse Transcription Polymerase Chain Reaction (RT-PCR) were categorized as "individuals with COVID-19", while those whose nasopharyngeal RT-PCR tests did not yield positive results throughout clinical follow-up were designated as "healthy controls" [20].

Molecular diagnosis of SARS-CoV-2

Diagnosis of COVID-19 infection was confirmed in the laboratory using a real-time reverse transcriptase polymerase chain reaction (RT-PCR) from nasopharyngeal and oropharyngeal swabs according to African CDC guidelines. Briefly, the swab samples were handled under a biosafety cabinet and placed in saline (0.9%), and RNA was extracted using the QIAamp® Viral RNA mini Kit (Qiagen) according to the manufacturer's instructions. The suspension was used for the RT-PCR assay for SARS CoV RNA. An extraction control, involving the amplification of an equine arteritis virus gene fragment using the "TIB MOLBIOL" kit was included to validate the diagnosis. The real-time RT-PCR assay was performed using a Superscript III RT-PCR kit Invitrogen in simplex, with primers and probes targeting the envelope coding for the E gene and the RdRP gene coding for RNA-dependent RNA polymerase (Tib-Molbiol kit). The reaction was performed on a 7500 real-time PCR System (Applied Biosystems). Samples were considered negative if the cycle threshold (Ct value) exceeded 36 cycles for the E gene and 40 cycles for the RdRP gene. In addition, a person was confirmed as a positive case if both nasopharyngeal and oropharyngeal swabs were positive, or if either was positive for SARS-CoV-2.

Measurement of plasma neopterin concentrations

All blood samples from all participants were centrifuged for 15 min at 3000 rpm cycles. Aliquots of sera were removed and stored at -80°C until analysis. To measure plasma neopterin, we used a commercial competitive neopterin ELISA (Neopterin ELISA, IBL International GmbH, Germany) and followed the manufacturer's instructions. 20 µl of samples, standards, and controls (with known concentrations) were measured in duplicate in wells of a microtiter plate coated with a goat anti-rabbit antibody. 100 µL of enzyme conjugate and 50 µL of neopterin antiserum were added to each well. The microtiter plate was covered with black adhesive foil and incubated in the dark for 90 min. After incubation, the adhesive foil was removed and the plate washed four times with 300 µL of diluted wash buffer. 150 µL of substrate solution were added into each well and the plate was incubated for 10 min. Finally, to stop the reaction, 150 µL of stop solution were added into each well.

To determine optical densities (OD) of the samples, plates were read using a microplate photometer (MultiSkan FC, Thermo Fisher Scientific K.K., Tokyo, Japan) with a 450 nm filter. Neopterin concentrations (in nmol/L) were deduced by fitting average OD on standard curves and were obtained for 325 plasma samples for which duplicates showed a coefficient of variation < 10%.

Statistical analyses

We used multivariate regression analysis in R to study the relationship between plasma neopterin concentrations and COVID-19 infection. We considered as predictors: COVID-19 status (class variable with two modalities: individuals with COVID-19 and healthy controls), sex (class variable with two modalities: male and female), age at evaluation (continuous variable), symptoms (class variables with five modalities: fever, cough, shortness of breath, headache and loss of smell and taste), associated comorbidities (class variable with four modalities: diabetes, cardiovascular disease, asthma and high arterial pressure) and time (class variable with three modalities: at day 0, day 10 and day 14). We log-transformed plasma neopterin concentration to obtain residuals that were normally distributed. We then removed the variables for symptoms and associated comorbidities because they were not easily comparable between groups and there

Table 1 Demographic and clinical characteristics of patients with COVID-19 and healthy controls

Characteristic	Control group	COVID-19 group
n	201	124
Demographic features		
Age (years), mean ± SD	35.2 ± 16.1	33.8 ± 14.6
Gender (m/f)	115/86	75/49
Symptoms, n (%)		
Fever	5 (2.4%)	17 (13.7%)
Cough	3 (1.4%)	7 (5.6%)
Shortness of breath	5 (2.4%)	13 (10.4%)
Headache	2 (1.0%)	8 (6.4%)
Loss of smell and taste	3 (1.4%)	11 (8.8%)
Comorbidity, n (%)		
Diabetes	6 (3.0%)	4 (3.2%)
Cardiovascular disease	7 (3.4%)	2 (1.6%)
Asthma	3 (1.4%)	2 (1.6%)
High arterial pressure	9 4.4%)	2 (1.6%)

was insufficient data. We then considered only one model adjusting for COVID-19 infection, age at assessment, sex and time as covariates. We performed post-hoc tests based on differences between the least squares means using one-way ANOVA. We further conducted a receiver operating characteristic (ROC) analysis to distinguish the COVID-19 group from the control group, and the area under the curve (AUC) was calculated. The 95% confidence intervals (95% Cls) were also calculated when appropriate, and a p-value < 0.05 was considered statistically significant.

Results

A total of 390 samples were collected at the CHRAB from March to August 2020. 15 samples with indeterminate PCR results, 10 samples with missing age and 45 samples with missing data between days 10, 14, 21 and 28 of monitoring were excluded. Ultimately, 325 samples were included and analyzed in this study.

Baseline clinical characteristics

Of the patients, 38% (n=124) were in the COVID-19 group, and 62% (n=201) were in the control group; 41.5% (n=135) were female and 58.4% (n=190) were male (Table 1). The mean of age of the patients was 34.72±15.62 (min-max: 10–77), the mean of age of the COVID-19 group was 33.8±14.6, and the mean of age of the control was 35.2±16.1. The mean of age of the study groups was not significantly different (p>0.05). Of the COVID-19 group, 39.5% (n=49) were female and 60.4% (n=75) were male, while 42.7% (n=86) were female and 57.2% (n=115) were male in the control group. Neither clinical symptoms, nor comorbidity rates differed significantly between the study groups (p>0.05).

Table 2 The influence of several factors on plasma neopterin concentrations

Variables	Estimate	F	*P*
Age (year)	0.004	2.87	**<0.001**
Sex	f: 0.013	0.24	0.810
COVID-19 status	Positive: 0.159	3.01	**0.004**
Days after infection	day 10: −0.344	-2.61	**<0.001**
	day 14: −0.601	-3.66	**<0.001**

The estimate associated with sex (f: females) was compared with males; estimates associated with COVID-19 status (Positive) was compared with Negative individuals; estimates associated with day after infection (day 10, day 14) was compared with day 0

F and P values of full linear models are presented with significant P-values in bold

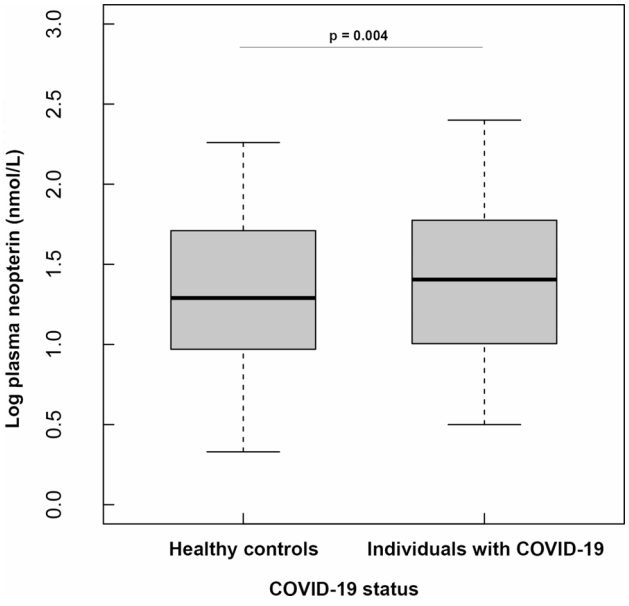

Fig. 1 Differences between plasma neopterin concentrations in relation to COVID-19

Neopterin concentrations and COVID-19 status

ROC analysis found that the best cut-off value of plasma neopterin levels for diagnosing COVID-19 patients was 38.85 nmol/L with 70% sensitivity and 82% specificity (AUC, 0.74 [0.69–0.82], p<0.05). We found higher neopterin levels in COVID-19 group (mean value 45.1 nmom/L (SD 19)) than the control group (mean value 33.8 nmol/L (SD 13)) (p=0.004, Table 2; Fig. 1). Plasma neopterin concentrations differed significantly with the time since infection, with the first day of sampling (mean value 45.1 nmol/L (SD 19)) showing higher plasma neopterin than day 10 (mean value 30.0 nmol/L (SD 2), p<0.001, Table 2; Fig. 2) and day 14 (mean value 25 nmol/L (SD 5), p<0.001, Table 2; Fig. 2).

We also found a significant increase in plasma neopterin concentration with age, with older individuals showing significantly higher neopterin levels (p<0.001, Table 2; Fig. 3).

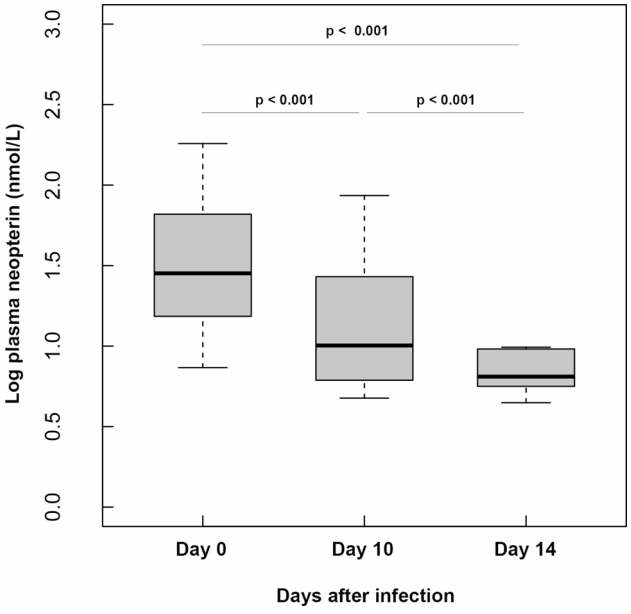

Fig. 2 Assessment of plasma neopterin concentrations at days 0, 10 and 14 post infection

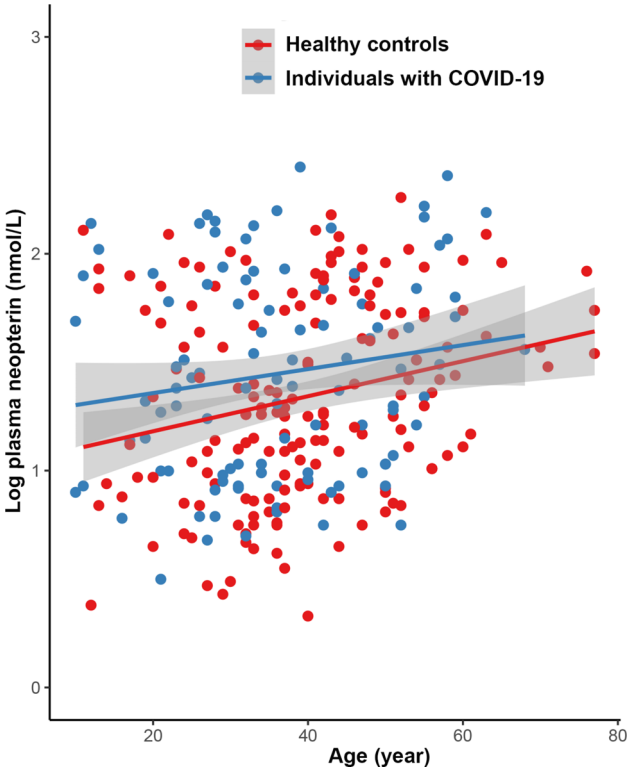

Fig. 3 The relationship between age and (log) plasma neopterin concentration (nmol/l), for individuals with COVID-19 and healthy controls. Predicted values are shown with 95% confidence intervals. Points represent the raw (log) plasma neopterin concentration values

Discussion

In the present study, we measured circulating plasma neopterin concentrations and investigated the cross-sectional relationship between plasma neopterin concentrations and COVID-19 infection. We found that plasma neopterin concentration were significantly higher in the COVID-19 group than in control group, and decreased gradually over time. We also found an increase in neopterin production with increasing age.

Higher neopterin concentrations in patients with COVID-19

The first finding is consistent with previous studies conducted during the COVID-19 pandemic and showed that neopterin levels increased in COVID-19 cases compared to healthy control [20, 21]. Serum neopterin levels in these studies were approximatively two times higher in severe patients than in patients with mild symptoms [20, 21], and four times higher than in healthy [22, 33]. This finding support the use of neopterin levels as an indicator of viral infections [17]. For example, a higher level of neopterin is associated with seasonal influenza [34], serum neopterin levels were correlated with increased viral load and mortality in HIV infected populations [17, 35], and serum neopterin level was an indicator of viral replication in hepatitis B and hepatitis [36].

In the present study, the cut-off value for diagnosing COVID-19 was set at 38.83 nmol/L, with a sensitivity of 70% and a specificity of 82%, indicating acceptable discriminatory power. Therefore, patients with a neopterin level above 38.83 nmol/L have a high probability of COVID-19 infection. This threshold is 2 to 6 times higher than those reported in previous studies comparing COVID-19 patients with healthy controls [20, 22], but is lower than the threshold used to identify severe COVID-19 cases [20, 37]. These results highlight neopterin's potential in diagnosing COVID-19 patients in the Central African region. Moreover, neopterin may serve as a biomarker for detecting the virus, monitoring disease progression, and predicting outcomes in COVID-19 patients. This biomarker can contribute rapidly and accurately in the identification of infected individuals according to the optimal value defined, particularly in cases with asymptomatic presentations where clinical diagnosis may be challenging.

Time course of neopterin decline

We found that plasma neopterin concentrations gradually decreased over time in patients with COVID-19. This finding is consistent with previous research [21] and suggests that the immune response is modulated during the course of the disease [38]. This may be associated with a reduction in viral load in patients receiving mechanical ventilation for COVID-19 [37]. Similar studies have shown that neopterin concentrations positively correlate

with circulating viral load in HIV-1 infected patients [39]. Furthermore, this finding highlights a dynamic response that may be important for disease monitoring and management. Monitoring neopterin levels over time mays also assist healthcare providers in assessing disease progression and evaluating the efficacy of therapeutic interventions. However, further large longitudinal studies in COVID-19 patients and controls are needed to confirm the consistency and clinical relevance of this observed trend.

Age-related variations in neopterin

Concentrations of plasma neopterin concentration increased with age, consistent with several other studies [40, 41]. This finding is in line with previous studies showed age as a strongest risk factor for COVID-19 infection. For example, the percentage of immunocompromised people in a population increases with age [42, 43], and the risk of death is higher in older adults than in younger people [44, 45]. Elevated neopterin in older individuals may also be associated with natural aging-related decline in immune system function (immunosenescence) [46]. This decline can result in a less effective immune response to COVID-19 [47, 48]. Elevated neopterin may reflect increased immune activation as the aging immune system attempts to respond to COVID-19. Additionally, this finding may also reflect the progressive activation of the immune system [40, 49]. This elevation may suggest chronic low-grade inflammation that often accompanies the aging process, and a further heightened state of immune surveillance and chronic immune activation, potentially increasing the risk of age-related undiagnosed diseases and impairing overall immune function [17]. This finding contributes to the increasing evidence that neopterin levels can indicate immunosenescence [40].

The observed variations in neopterin levels may be due to limitations in our study. The study participants experienced milder cases of COVID-19 compared to those in previous studies where neopterin was investigated as an indicator of disease severity and prognosis. Additionally, most of our participants were asymptomatic. Finally, the study was conducted in a region where endemic diseases such as malaria, and other parasitic, bacterial, and viral infections might increase the baseline of neopterin [50].

Conclusion

Our findings contribute to the growing understanding of neopterin levels in sub-Saharan Africa.

The fact neopterin is a marker of an unspecific inflammatory process make it necessary to establish baseline level of neopterin as an essential step in the assessment of population health, the diagnosis of pathologies such as COVID-19, malaria, and other endemic parasitic, bacterial, and viral infections in sub-Saharan Africa. While our study indicates potential utility, the sensitivity and specificity results suggest that neopterin, in its current application, may not yet be a reliable biomarker for COVID-19 detection in this region. Further studies are necessary to thoroughly analyze and validate the role and efficacy of neopterin as a biomarker for COVID-19 in sub-Saharan Africa.

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Author contributions

SED, JBLD, and BN Conceived and designed the study. SED and DOE designed the lab works. SED and YON performed the neopterin analysis. CNMMN, SLOL, and JBLD designed the survey on blood collection. SED wrote the original draft and data analysis. All authors contributed to the article and approved the submitted version.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Gabonese National Research Ethics Committee (permit number: 003/2020/CNER/SG/P). Additionally, informed consent was obtained from all participants included in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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